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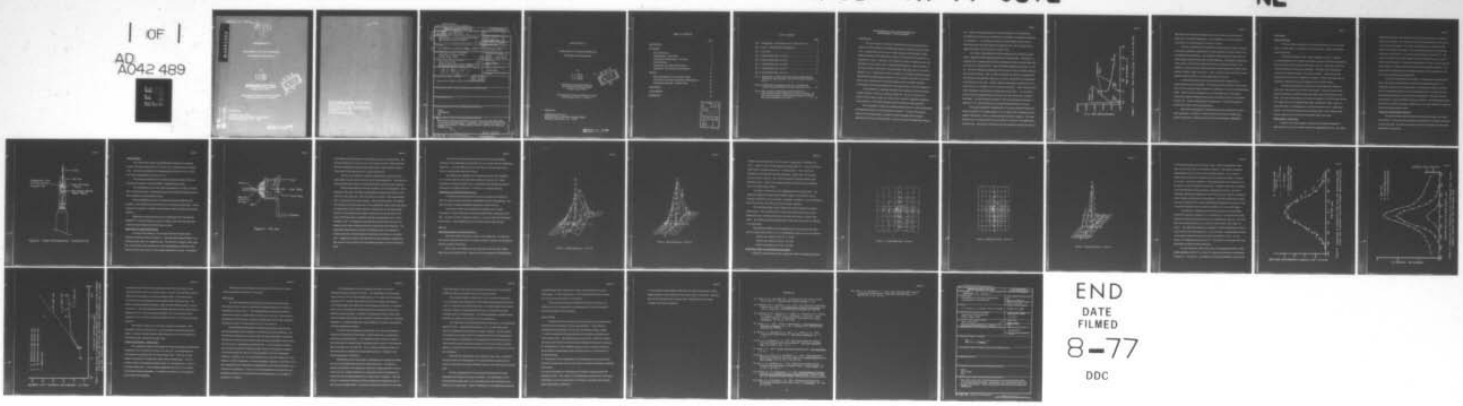

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Report BMER-127-77

Measurements in the Laser Irradiated Eye

(Development of Microsensors)

by

A. J. Welch
L. D. Forster
L. A. Priebe

Biomedical Engineering Laboratory
The University of Texas at Austin
Austin, Texas 78712

Final Report for Contract F44620-76-C-0066
for the period 1 Apr 76 - 31 Mar 77

Prepared for

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Micro fiber optics and combination sensors have been fabricated which can measure light distribution, temperature, and electrical activity in the in vivo ocular fundus. These sensors have been tested in the rabbit and monkey eye.

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MEASUREMENTS IN THE LASER IRRADIATED EYE
(DEVELOPMENT OF MICROSENSORS)

BACKGROUND

The use of lasers as clinical and industrial tools has stimulated much interest in the description of the interaction between light and biologic tissues (1,2). Intense light sources such as lasers damage or alter biologic tissues primarily because of the absorption of light in the tissue and the conversion of light energy to heat. Clinically, this alteration provides a valuable tool for the treatment of many diseases and a valuable surgical device. Many diseases of the eye have been successfully treated clinically with laser light in the visible spectrum (3,4). Although the success of lasers as a treatment modality has been demonstrated for pathological conditions such as diabetic and sickle cell retinopathy, some complications of photocoagulation such as choroidal ischemia, hemmorhage and neural layer destruction have been noted in clinical treatment (3).

The possibility of accidental exposure from operational and R and D laser systems creates a concern for personnel safety (2). The eye, because of its ability to focus light to small spot sizes and its inability to regenerate destroyed neural tissues, is the primary biologic organ of interest for safety purposes.

The assessment of safety and treatment effectiveness is currently determined by subjecting animals to various exposure conditions. To reduce the required number of animal experiments, models have been developed which compute temperature rise due to light absorption in the eye and predict temperature increase

for a variety of exposure durations, image distributions, and wavelengths. To establish the accuracy and credibility of the models, experimental validation is necessary (7). The prediction accuracy of these models and the critical model parameters are being tested and identified with a set of *in vivo* and *in vitro* experiments at The University of Texas (8,9).

The critical measurements in these experiments are the transient temperature response and the intensity profile of the retinal image. Temperature is measured with a copper-nickel junction thermocouple which is vacuum deposited on the tip of a quartz fiber (10). The tip diameter generally varies from 10 to 20 μm. The direct absorption properties of the junction provide an indirect measurement of light intensity. That is, light that strikes the junction is absorbed and the resulting heat produces a rapid rise in junction voltage which is proportional to the intensity of the light. Figure 1 shows the temperature measured 10 μm in front of the P.E. in response to a 0.01 second laser pulse. Direct absorption and tissue temperature components are clearly seen in this figure. As the probe is retracted into the pigmented layer of the eye, the direct absorption component diminishes. For most exposure conditions, the direct absorption component is less than 0.5°C when the probe is in the light absorbing tissue of the eye.

The *in vivo* temperature measurements in the laser irradiated monkey eye require placement of the thermocouple in a 10 μm thick layer of pigmented tissue (pigment epithelium), which is located directly behind the receptors. This layer absorbs most of the light entering the eye and thus is the most susceptible layer to laser injury. We assume, based upon the heat conduction equation for the eye,

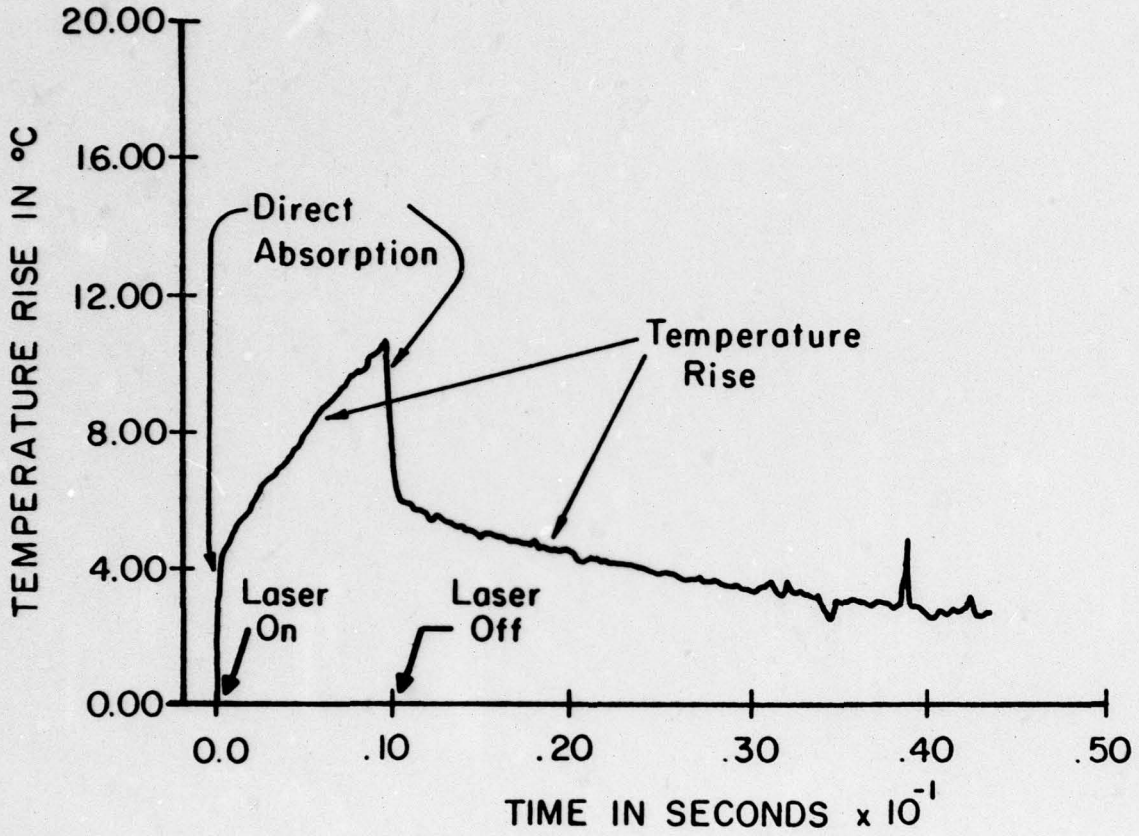

Figure 1. Temperature - time history 10 μm in front of the P.E.

that the point of maximum temperature rise occurs in the center of the laser beam and in the pigment epithelium. This assumption can only be verified if the position of the thermocouple in the eye is known.

We have considered the development of several multiple purpose sensors to provide simultaneous measurements of light intensity and probe position with the temperature measurement. By combining a thermocouple and light probe we could directly measure temperature and light intensity. Simultaneous comparison of the effect of light directly striking both sensors would provide data on the validity of intensity profiles measured using the thermocouple and provide a more sensitive sensor of light at the retina. Also, a more sensitive light sensor could measure intensity profiles in the absorbing layers of the eye.

A combination probe which could provide position information is a thermocouple and micro-electrode. The distinct electrical activity of the various layers of the retina with light stimulation is reflected in the local electroretinogram (ERG). Phase reversal of components of the local ERG occur at the bipolar, receptor and pigment epithelium layers due to the dipole nature of the electrical activity (11). Using a thermocouple-electrode probe, it would be possible to locate these layers with the mocroelectrode.

Although the development of these probes has been prompted by a particular experiment, we believe a variety of uses will be found for probes that possess temperature, light and electrical activity measurement capabilities.

PROCEDURE

Probe Fabrication

Fiber optic probe substrates are made by drawing down 0.05 inch glass rods in a probe puller to a diameter of 10 to 20 microns. Smaller tip diameters are also obtained.

A reflective coating of metal, either aluminum or silver, is applied by vapor deposition to the walls of the probes to insure (1) efficient light piping, and (2) coupling of light into the probe only at the tip. Deposition of silver by a chemical reduction process was attempted. Difficulty was encountered in achieving a completely opaque, pin hole free coating. Copper was electrodeposited over the silver to complete the coating. Probes of this type were not completely satisfactory due to the bulkiness of the copper coating.

A rotating probe holder was made for the vapor deposition chamber to allow even vapor coating on all sides of the probes. Preliminary results with aluminum coatings give efficient light pipes which are nearly opaque on their walls. The next and possibly final stage in development of the fiber optic probes is to apply silver in the rotating probe holder, followed by copper, either by vapor deposition or electrodeposition; if the latter process can be sufficiently refined. The silver would provide the high reflectivity, and the copper would provide opacity and also protect the relatively fragile silver film.

Thermocouple - light probes

Standard 1 mm glass pipettes were drawn to an outer tip diameter of approximately 50 μm and an interior diameter of approximately 30 μm. An annular

thermocouple junction was formed at the tip by vapor deposition of nickel and copper on the pipette. First a 2000 Å nickel film was deposited on the tip and on one side of the pipette. The nickel was annealed and a nickel lead was attached to the side of the probe. The tip was placed against a mask and the probe was covered with a 0.7 μm thick insulating layer of parylene C®. The mask was removed and a 2000 Å copper film was deposited on the tip and non-nickel side of the probe. Following the attachment of a copper lead on the side of the pipette, the entire probe was coated with the parylene insulation. The same general procedure has been reported in detail for the construction of 10-20 μ quartz substrate thermocouples (12).

In order to sense light with the probe, a viscous optical coupling fluid (Dow Corning 200 Clear Silicone) was forced into the pipette using a 1 cc hypodermic syringe and a 26 gauge needle. A photodetector (Bell and Howell 529-2-5) was placed against the end of the pipette and the coupling fluid was continuous from the probe tip to the face of the detector. The probe detector was mounted in a pin vise holder as shown in Figure 2. The thermocouple leads, located on the shank of the probe outside the pin vise were soldered to respective nickel and copper amplifier leads.

Combination Thermocouple-Electrode

The pipette thermocouples formed with the above procedure were filled with 3M KCl. A silver wire was inserted in the large end of the probe and sealed in place with wax. The probe was placed in the pin vise holder and leads were attached to the amplifiers.

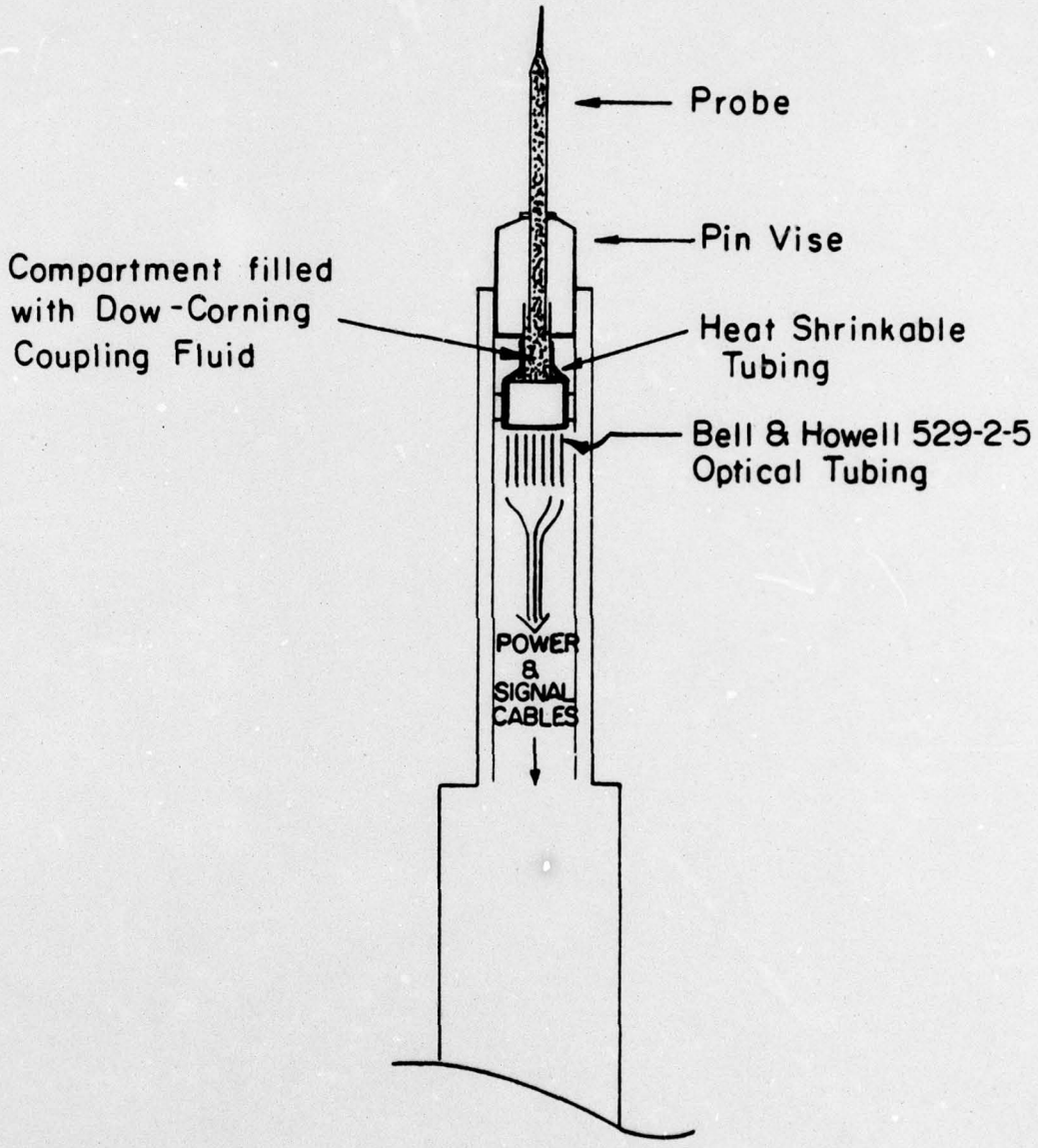

Figure 2. Probe–Photodetector Configuration

Instrumentation

The thermocouple signal was differentially compared to a reference junction that was maintained at 37°C ± 0.05°C by a proportionally controlled oven. The system bandwidth for the thermocouple system was DC to 10 kHz. The system measurement error was ± 0.2°C.

The electrode preamplifier was a Grass Instruments Model P18B micro-electrode DC Amplifier whose bandwidth is approximately 300 kHz.

The photodetector for the light probe measurements is a Bell and Howell 529-2-5 Optical Detector, a photodiode-operational amplifier combination which has a bandwidth of approximately 60 kHz.

These preamplifiers are fed to an eight channel Clevite/Brush Chart Recorder. The signals are also monitored on a two channel oscilloscope. Analog signals are recorded on a Sangamo tape recorder for later digitizing and computer analysis.

Experimental control and timing are accomplished with a Devices Ltd. Digitimer®. A Vincent Associates shutter is used to pulse the laser beam from a Spectra Physics Model 166 4W CW argon ion laser.

Temperature and Light Measurements

The probes were tested in a two layered cell that has been used to validate the thermal model (see Figure 3). The laser beam passed through a non-absorbing water layer into a pigmented gal. The gel was a mixture of water, Agar gel, and sufficient black drawing ink so the light absorption coefficient of the mixture was the same order as in the pigment epithelium of the eye. The mixture

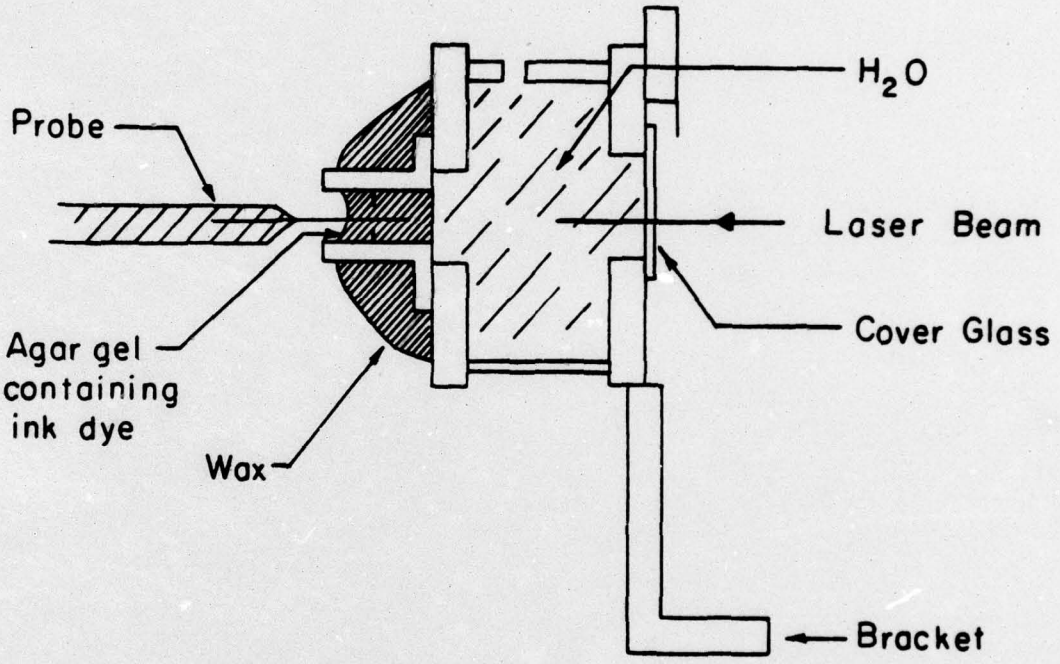

Figure 3. Dye Cell

was heated and then allowed to cool and gel face down on a glass slide. The gel was mounted on the cell as shown in the figure and held in place with wax. The front compartment was filled with water under a slight positive pressure which held the gel stationary when a probe was inserted.

The cell was mounted on a platform with the front cover glass at the same position as the cornea of the eye for an animal preparation. The probe was driven through the gel until it was observed in front of the gel-water interface.

Probes were inserted into the gel by means of a micromanipulator. After insertion of the probe, its depth was adjusted by observing the position of the probe tip in the water cell. When the probe was visible, it was approximately 300 μ in front of the gel-water interface. With the probe visible, the platform was adjusted until the probe tip was in the approximate center of the laser image. The light sensor and the direct absorption characteristic of the thermocouple during 10 msec shuttered laser pulses was used to position the probe in the center of the beam (point of maximum intensity and temperature due to direct absorption) (8). By rotating the cell about the vertical axis of the eye, the relative retinal image irradiance profile and image radius were measured. The temperature measurement was readily quantified because the temperature-response time due to direct absorption was faster than that due to conducted heat. Temperature changes in the probe due to direct absorption appeared as a step increase with the start of the light pulse and again at the end of the light pulse.

The probe was retracted toward the gel until the direct absorption component of the temperature measurement was much smaller than the temperature component. From this reference point in the center of the laser beam and in the gel, measurements were made radially.

The platform was equipped with stepping motors that had a resolution of 1.6 microns per step at the gel/water interface of the dye cell. Axial adjustment of the probe position was accomplished with a Burleigh Inchworm^{R} piezoelectric microdrive that had a resolution of 0.5 microns per step.

Temperature and Electrical Measurements

A combination thermocouple-electrode was inserted into the dye cell using the same procedure outlined for temperature and light measurements. The laser was used to induce measurable temperature rises in the cell.

An electrical signal was introduced by installing a pair of wires connected to a signal generator into the front (water-filled) compartment of the cell. In order to insure a conductive solution, 0.9 gm % saline was substituted for the water in this compartment and for the water used to make the gel.

RESULTS

Light Measurements in the Monkey Retina

Fiber optic probes have been inserted in the monkey eye. By scanning the animal through the laser beam it has been possible to produce two dimensional intensity profiles of the retina.

Beam scans in two dimensions have been taken with fiber optic probes both inside and outside the eye. Figures 4 and 5 are perspective representations

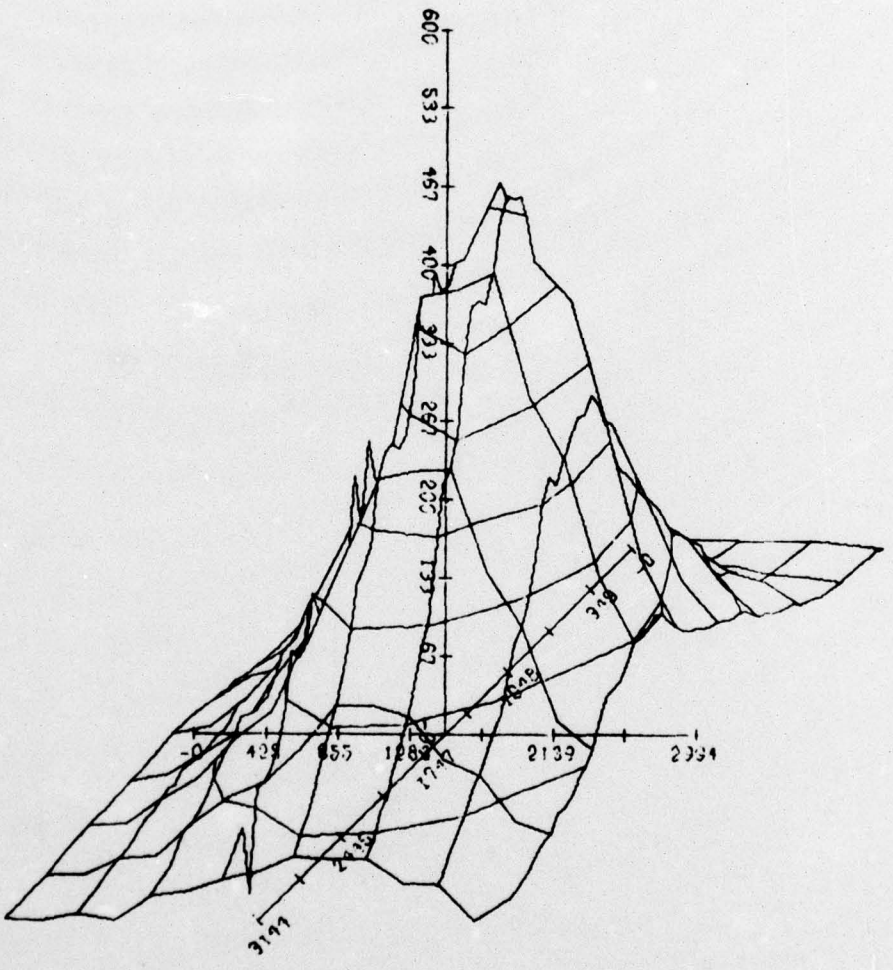

Figure 4. Corneal Beam Scan 10-22-76

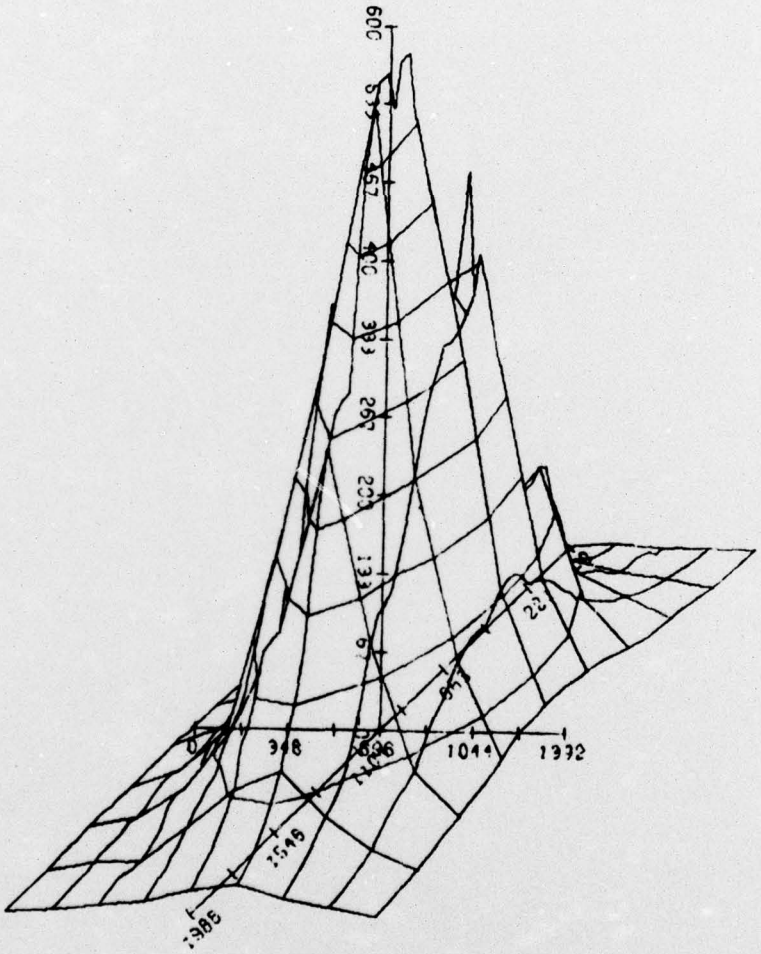

Figure 5. Retinal Beam Scan 10-22-76

of beam scans at the cornea and at the retina, respectively, for Monkey 10-22-76. Figures 6 and 7 are the respective contour plots (i.e., lines of constant light intensity, projected onto the x-y (retinal) plane). The x and y axes correspond to vertical and horizontal directions, respectively, on the retina, and are scaled in microns. The z axes are scaled in arbitrary relative light intensity values. Figure 8 is a perspective plot of the beam scan for Monkey 10-27-76, taken inside the eye.

The sizes of the beams can be determined from the contour plots. The distance between $1/e^2$ points (14% of peak) in Figure 7 are 1271 μm in the x direction and 927 μm in the y direction. Similarly, in Figure 6, the $1/e^2$ diameters are 2373 μm in the x direction and 2776 μm in the y direction.

The fiber optic probes used in these scans had tip diameters of approximately 30 μm. The insertion site for all the scans inside the eye was in the paramacular area, and the probe tip was well into the vitreous, in front of the retina. The average number of sample points in the scan rasters was 50 (vertical) x 10 (horizontal).

The exposure duration for each sample point in the scans was 250 msec, and the incident laser power at 514.5 nm wavelength for the scans was as follows:

Corneal scan monkey 10-22-76: 17.4 nW

Retinal scan monkey 10-22-76: 52.3 nW

Retinal scan monkey 10-27-76: 64.4 nW

Combination Light and Temperature Measurements

Preliminary data were taken with combination light and temperature sensors

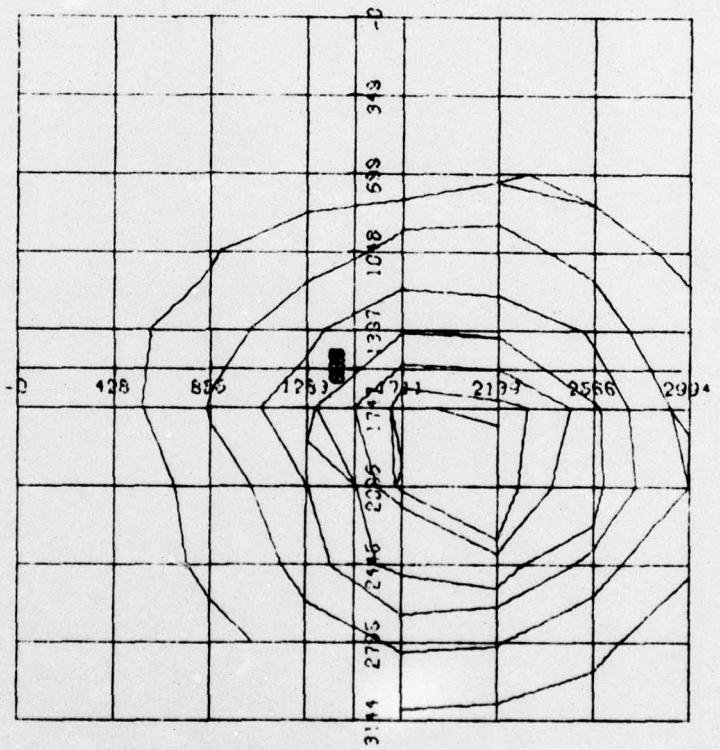

Figure 6. Corneal Beam Scan 10-22-76

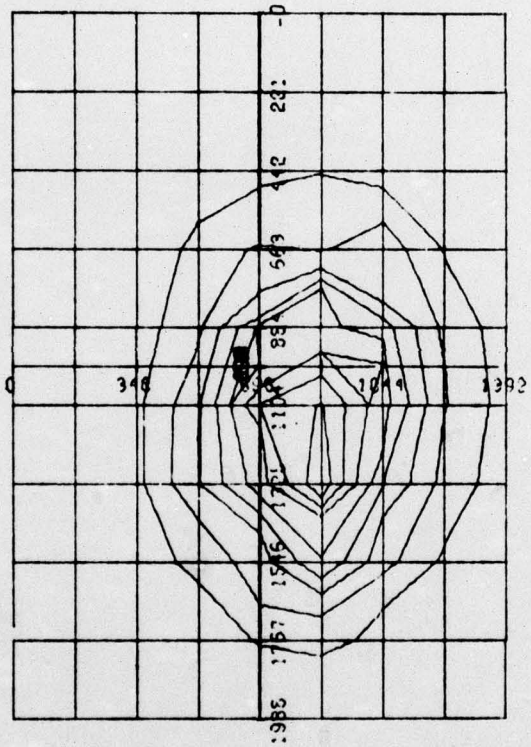

Figure 7. Retinal Beam Scan 10-22-76

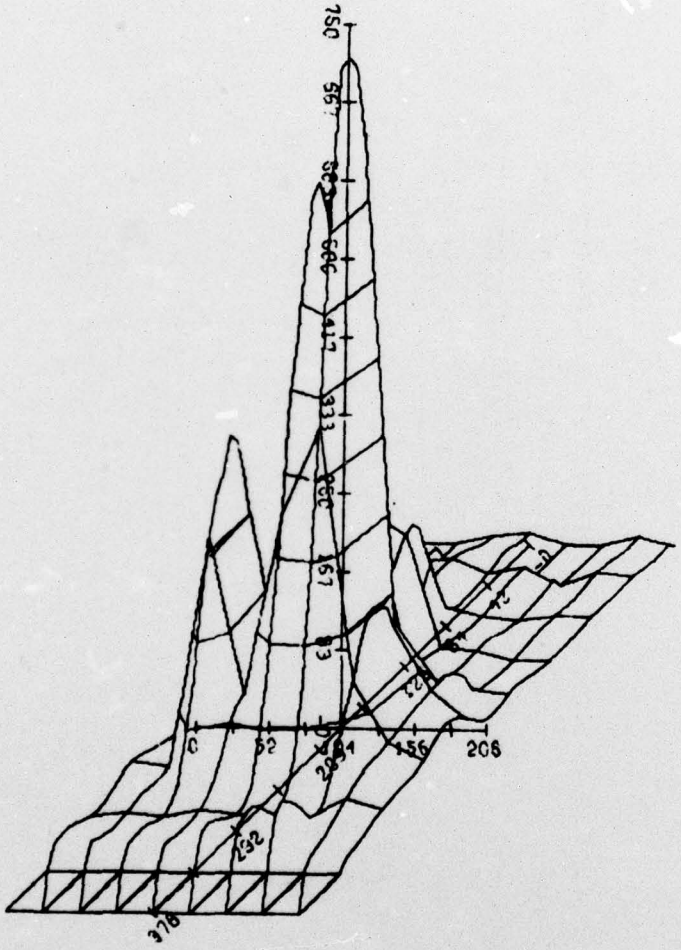

Figure 8. Retinal Beam Scan 10-27-76

in two rhesus monkey eyes and two dye cells. Light and temperature scans from one of the dye cells are presented in Figure 9. The probe is positioned approximately 300 μm in front of the gel-water interface. A 0.01 second duration laser pulse with a repetition rate of one pulse per second is directed on the cell. The cell is rotated 25 μ horizontally between each light pulse so the probe is scanned through the laser beam. A portion of the light is absorbed in the thermocouple junction producing a temperature increase. This direct absorption measurement samples the beam and provides an indirect measurement of the intensity profile with the thermocouple. The direct light probe measurement made simultaneously with the thermocouple measurement has been fit with a gaussian distribution with a standard deviation of 50.9 μm in the figure.

A comparison of temperature scans with the probe near the gel/water interface for exposure durations of 0.03 second and 1.0 second with the simultaneously sampled light probe beam scan are illustrated in Figure 10. The laser beam had an indicent power of 10.5 mW and a beam radius ($1/e^2$ radius) of 102 μm. The temperature profiles are measured at radial displacements relative to the center of the laser beam scan. At 0.03 seconds, the temperature increase in the center of the beam at the end of the laser pulse is 10.5°C. At one second, the temperature increase is 13°C. The spread of the profiles with time illustrates the effects of heat conduction.

For the above data, plots of the ratio of the temperature profile to light profile diameters at the 1/2, 1/3 and $1/e^2$ points of the profiles are illustrated in Figure 11. For example, the diameter at which the temperature was one-half

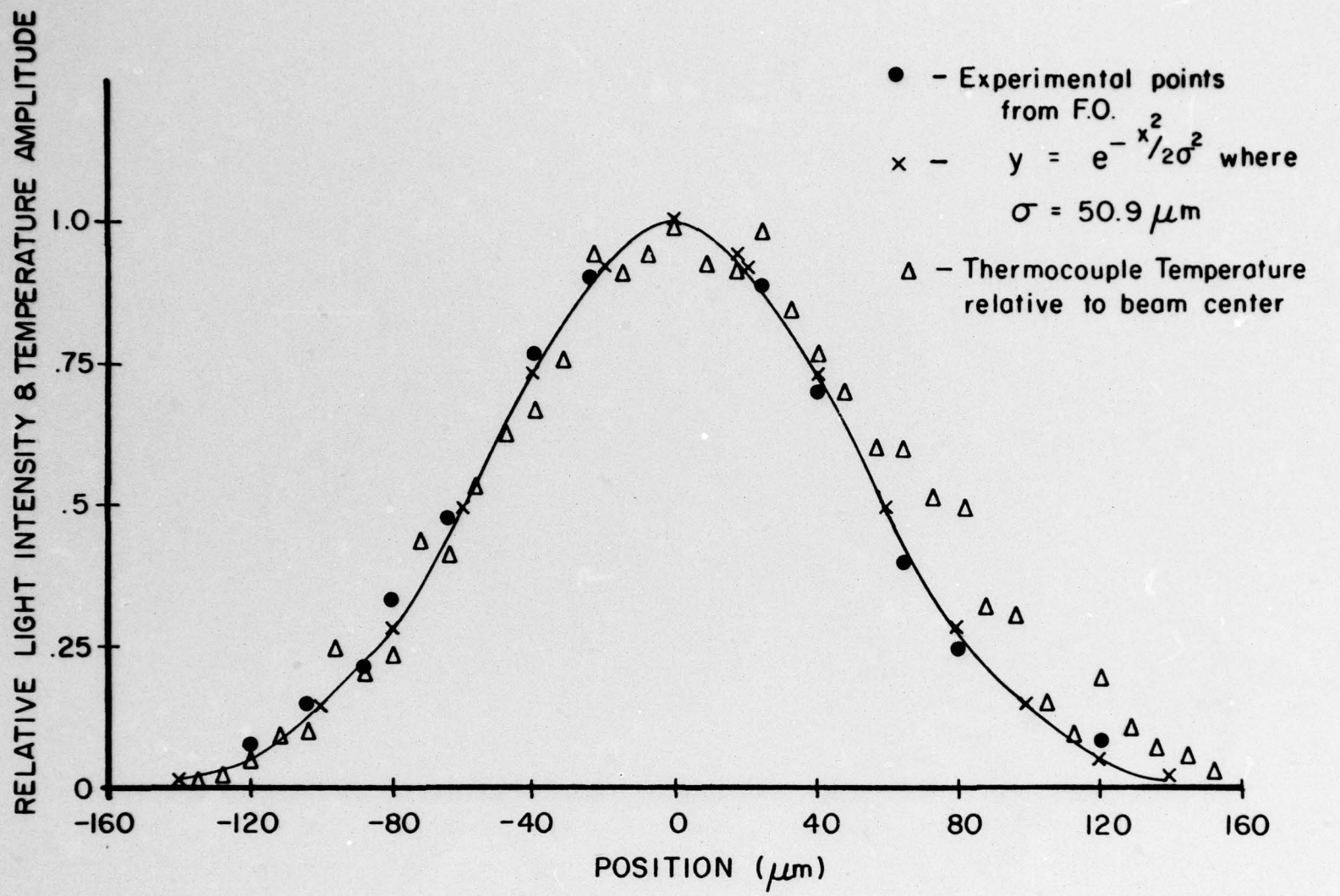

Figure 9. Comparison of combination fiber optic and thermocouple beam scans with a gaussian curve with standard deviation of 50.9 μm.

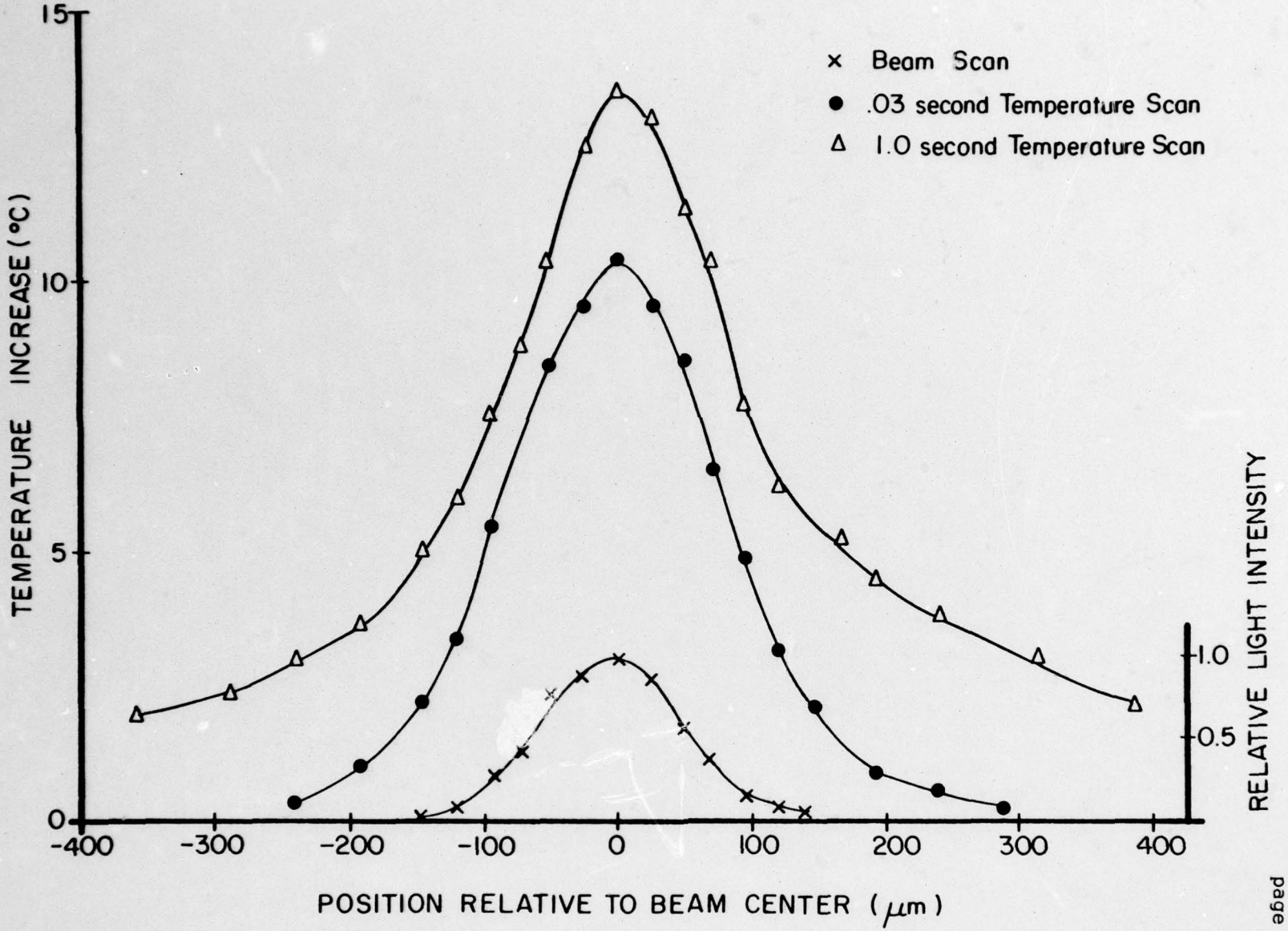

Figure 10. Comparison of temperature scans of .03 second and 1 second exposure duration with fiber optic beam scan.

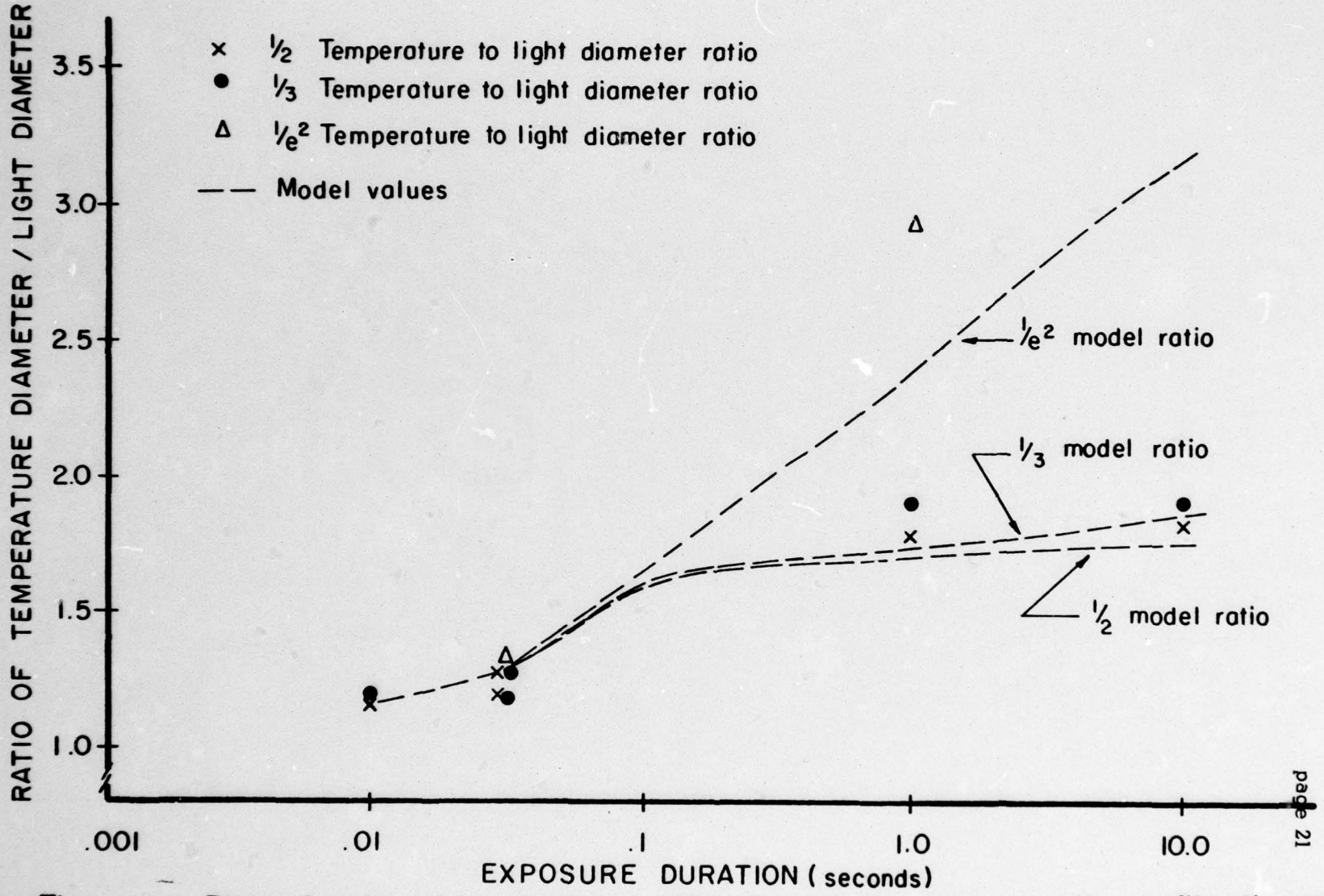

Figure 11. Plots of ratios of temperature profile diameters to light intensity profile diameters at ½ peak, ⅓ peak, and $1/e^2$ times peak values. Model predicted values are shown along with preliminary data points.

its peak value was divided by the diameter at which the light intensity was one-half its peak value as a function of time. Note that the temperature profiles spread with time relative to the fixed intensity profile. The thermal model was used to predict temperature for the measured light intensity profile. The ratios of model temperature diameter to light intensity diameter appear as curves in Figure 11. The model-predicted peak temperatures for the given light intensity profiles are 20.5°C for the 10 second exposure, 19.4°C for the one second exposure, 13.4°C for the 0.03 second exposure, and 10.1°C for the 0.01 second exposure.

Not shown in Figure 11 are the ratios of beam scan diameters. With the probe in the non-absorbing layer, direct absorption intensity profiles were within 1.2 percent of light intensity profile diameters over the entire profile for data from two dye cells and two monkey eyes.

Combination Electrode - Thermocouple

The combination pipette thermocouple-KCl electrode measured simultaneous temperature rise and electrical activity in the dye cell. The temperature rise was induced by irradiating the cell with the Argon laser. There was a small amount of crosstalk of the electrical signal onto the thermocouple. For a 40 millivolt signal at the electrode amplifier input, the crosstalk was 5 μ volts on the thermocouple input. This crosstalk represented less than 0.25°C artifact in the thermocouple measurement. For signals on the order of a few millivolts, the crosstalk was negligible.

Although a triple measurement was not made with this probe, the KCl inside the pipette was observed to pipe light.

DISCUSSION

The fiber optic permits measurement of low level light at the retina. The probe provides a means of measuring the intensity profile at the retina as illustrated in Figures 5 and 7. With smaller probes it should be possible to measure point and line spread functions for the eye. By using a more sensitive photodetector with the probe, we believe the fiber optic can be used to measure the percent of light at various depths in the P.E. and choroid.

The simultaneous measurement of light and temperature signals makes possible meaningful direct comparison of conduction and light absorption. The actual beam distribution at the axial position at which temperature is measured provides data which are not available using only the thermocouple as both a beam scan device and a temperature measurement tool. For long exposure durations with the probe positioned in absorbing media, the direct absorption of radiation in the thermocouple may only be a small percentage of the total temperature increase. Typically, for a 10 second exposure in the eye which results in a 20°C increase in fundus temperature, the direct absorption component is less than 0.5°C. The components may be separated into temperature rise and direct absorption, but only with some difficulty. A beam scan for these exposures taken from the direct absorption component is not reliable since the system noise is of the order of a few tenths of a degree.

A few thermocouple scans and separate fiber optic scans have measured the beam intensity in the retina. The thermocouple was positioned 100 μm or more in front of the absorbing layers of the fundus and short duration exposures (0.01 seconds) were employed to achieve reliable measurements. As the thermocouple was withdrawn toward the pigment epithelium, the measurement of the scan became less reliable because of conducted heat which artifactually spread the profile. Although good agreement between separate fiber optic and thermocouple profiles was obtained at positions anterior to the pigment epithelium, no reliable comparisons were possible for the probe at the position of highest temperature increase.

The fiber optic-thermocouple combination could solve this problem by allowing the simultaneous measurement of light distribution and temperature very near the absorbing layers. The combination probe verifies the ability of the thermocouple to measure image distribution near the retina of the absorbing layer in the dye cell (see Figure 9). The 1.2 percent agreement between the two scans from the simultaneous measurement provides a validation of the thermocouple beam scan method.

Simultaneous scans also provide a meaningful direct comprison between the size of the temperature field and the light distribution. A comparison between the diameter of the temperature field and the image diameter at various points provides a simple comparison of conduction between the experimental preparation and the model predictions for various times of exposure. The data indicate a reasonable fit between model and experimental temperature field to light intensity diameter ratios. The effects of boundary conditions on the model,

blood flow effects in the model and experimental preparation and absorption parameter effects are possible with this combination probe.

Since pipettes pulled to diameters of 4 μm or less have been filled in our laboratory with coupling fluid, much smaller thermocouple-light probes may be constructed using these techniques. The thermocouple may be used to locate the position of peak temperature relative to the interface between absorbing and non-absorbing media. The light measurement capability makes possible the location of the interface for this measurement.

The combination electrode-thermocouple constructed on a small diameter pipette will allow temperature measurements in the non-absorbing neural layers for comparison with rate process models of damage. The localization of potentials in the retina will allow accurate location of the thermocouple in the neural layers. Measurements of temperatures at known positions in the retina will allow more accurate descriptions of the extent of axial and radial damage in the neural layers of the retina when coupled with microscopic evaluation techniques.

Since the KCl electrode was also observed to pipe light, it should be possible to mask the thermocouple with a highly reflective coating such as silver and thus reduce the direct absorption artifact in the thermocouple measurement.

Several configurations for the simultaneous measurement of light, temperature and electrical activity are possible. One possibility is a KCl electrode-thermocouple with a long salt bridge which takes advantage of the ability of KCl to pipe light. Another configuration is the combination light pipe

and thermocouple with masked silver-silver chloride deposited over the thermocouple. A third alternative is a two barralled pipette with one barrel as a light pipe and the other a KCl electrode.

Other possible electrode configurations include the insertion of a platinum wire into the pipette for the measurement of electrical potentials, gas partial pressures and temperature.

CONCLUSIONS

Combination probe measurements in living eyes and dye cells present several distinct advantages over previous techniques. Some important measurements made possible with the use of combination probes include:

1) Direct measurement of light distribution at the pigment epithelium layer in the ocular fundus. This measurement may be used to validate the thermal and rate process models for the prediction of damage following laser exposure.

2) Measurements of light absorption in vivo in the eye should be possible. Axial absorption measurements will be of great value to the improvement of the thermal model.

3) The position of the thermocouple may be determined more accurately than previously possible with the aid of the light or electrical measurement capability of the probe.

4) Direct measurement of conduction may be made by comparing light and temperature scans. The "spread" of the temperature field relative to the image distribution at a given axial position in the fundus is possible with the light probe-thermocouple combination.

5) The electrode-thermocouple combination will allow measurement of temperature fields at a known position in the neural layers of the fundus. Measurement of the temperature field in these layers will provide data for model validation and damage prediction.

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Micro fiber optics and combination sensors have been fabricated which can measure light distribution, temperature, and electrical activity in the in vivo ocular fundus. These sensors have been tested in the rabbit and monkey eye.

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